

**REMARKS**

Claims 1-4, 7, 8, 10-32, 39 and 40 are pending. Non-elected claims 33-38 and 41-45 are canceled. Claim 1 is amended.

Claims 1-4, 7, 8, 10-32, 39 and 40 were rejected under 35 USC §103(a) as being unpatentable over Altieri. Favorable reconsideration of this rejection is earnestly solicited.

In the Office Action dated February 3, 2005, the Examiner highlighted that certain varieties of hybrid corn starch has more than 80% amylose. The Examiner stated that amylo maize would read on the claimed maize.

Claim 1 has been amended to specify ordinary maize.

It is true that varieties of hybrid corn are available which have a higher amylose content than ordinary maize. However, it is common practice to identify terms such as "maize starch" as ordinary corn starch, whereas varieties having a higher amylose content are always identified in the literature with terminology such as "amylo maize" or "high amylose starch." This can be seen by reference to column 10, Example 1 of Altieri which refers to "corn starch" as having 25-28% amylose and to "high amylose starch such as Hylon V and VII (column 10, lines 31-35 and 51-55).

In addition, EP 0 696 611, cited on page 3 of the present specification, and incorporated by reference, also distinguishes "corn starch" from high-amylose starch (see page 3, lines 6-8 thereof).

The term "ordinary corn starch" is used in the literature, as can be seen, for example, in the attached article by Jacob A. Rendleman, Jr. at page 174, Table 2. Furthermore, applicants attached pages 417-418 of an article by Stanley A. Watson showing that the word "corn" and "maize" are synonymous.

The specific starches set forth in claim 1 of the present application have an amylose content which is well below the 45 wt% required by Altieri. In the Office Action, the Examiner appears to suggest that the claims must be amended to state less than 45 wt% amylose. Applicants respectfully disagree.

Altieri teaches that at least 45 wt % amylose is required in its invention. This is a teaching away from the starches set forth in claim 1 of the present application. The point of novelty of applicants invention is not only the specific starches, but also the claimed cell size, cell size distribution and intrinsic viscosity in DMSO. Altieri does not disclose these features, nor would Altieri suggest or inherently provide these features as Altieri teaches away from the present invention. According to the present invention, it has been discovered that the desirable properties in terms cell size, distribution, closed cell structures and densities can be achieved by properly controlling the intrinsic viscosity within a relatively narrow range. Thus, the invention is not taught or suggested by Altieri.

For at least the foregoing reasons, the claimed invention distinguishes over the cited art and defines patentable subject matter. Favorable reconsideration is earnestly solicited.

Amendment  
Serial No. 09/784,707  
Attorney Docket No. 030229

Should the Examiner deem that any further action by applicants would be desirable to place the application in condition for allowance, the Examiner is encouraged to telephone applicants' undersigned attorney.

If this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. The fees for such an extension or any other fees that may be due with respect to this paper may be charged to Deposit Account No. 50-2866.

Respectfully submitted,

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Attachments: Petition for Extension of Time

“Corn and Sorghum Starches: Production” by Stanely A. Watson

“Hydrolytic action of  $\alpha$ -amylase on high-amylose starch of low molecular mass”  
by Jacob A. Rendleman, Jr.

EP 0 696 611

## Hydrolytic action of $\alpha$ -amylase on high-amylose starch of low molecular mass

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High-amylose starches of low average degree of polymerization (dp 61–71), formed as fine granules by interaction of *Bacillus macerans* cyclodextrin glucanotransferase with  $\alpha$ -cyclodextrin (CD) at 2–70 °C, are highly insoluble in water and not gelatinizable under normal cooking conditions (100 °C). Samples of CD-derived starches, both cooked and uncooked, were subjected to hydrolysis *in vitro* by human salivary  $\alpha$ -amylase at 37 °C under conditions chosen to resemble those in the human intestinal lumen. Released low-molecular-mass saccharides were determined quantitatively by HPLC and the results compared with those from similar studies with natural starches. Among uncooked starches, CD-derived starch showed very low reactivity towards  $\alpha$ -amylase, along with potato starch and a high-amylose hybrid corn starch (64% amylose). Cooking greatly enhanced reactivity of natural starches, but only moderately increased reactivity of CD-derived starches. Susceptibility to hydrolysis of cooked starches increased in the following general order: CD-derived starch ( $\approx$  100% amylose) < 100% corn amylose (isolated by the butan-1-ol method) < hybrid high-amylose corn starch (64–66% amylose) < waxy maize starch (99–100% amylopectin) < ordinary corn starch ( $\approx$  25% amylose) < potato starch ( $\approx$  25% amylose).

### Introduction

Starch is the most extensively used polysaccharide in food preparations and serves as the principal nutrient in the diet of the Western world. Digestion of starch by humans occurs primarily in the small intestine where two separate families of carbohydrases (enzymes that hydrolyse carbohydrates) are found [1]. One family is introduced into the intestinal lumen and is represented by  $\alpha$ -amylases (salivary and pancreatic). The other family is immobilized in the brush-border membrane and includes disaccharidases (such as maltase, lactase and sucrase), trisaccharidases and glucoamylase (amyloglucosidase). Glucoamylase liberates glucose specifically from the non-reducing end of malto-oligo-

saccharides (with greatest affinity for chain lengths of 5–9 glucose units [1]). Pancreatic  $\alpha$ -amylase predominates in the small intestine and is located largely in the lumen.  $\alpha$ -Amylase specifically catalyses the hydrolysis of  $\alpha$ -1,4 glycosidic linkages in starch. Amylose, a linear  $\alpha$ -D-glucan, is hydrolysed to maltotriose, maltose and a small amount of free glucose. Hydrolysis of amylopectin, the highly branched form of starch, likewise yields maltotriose, maltose and a small amount of glucose; however, because of the numerous enzyme-resistant branch points,  $\alpha$ -limit dextrins ( $\alpha$ -1,6-glucosides with 3–6 glucose units) are also formed. Action by  $\alpha$ -amylase on maltotriose to produce maltose and glucose is very weak and, therefore, of minor importance in the overall production of maltose and glucose. Maltose appears to be totally resistant to the enzyme. Because only monosaccharides are absorbed through the intestinal wall, maltose must first be hydrolysed to glucose by membrane-bound carbohydrases in the brush border. These same brush-border enzymes contribute to the breakdown of other malto-oligosaccharides. Glucose is absorbed at the rate of 5–20 g/h in the adult duodenum, and at 25–40 g/h in a 45-cm segment of the jejunum.

Although dissolved or gelatinized forms of natural starches react rapidly towards  $\alpha$ -amylase, reaction rates of raw or granular forms are much slower and vary according to the source of starch. Leach and Schoch [2] found the following order of increasing resistance of granules towards  $\alpha$ -amylase: waxy maize < tapioca < waxy sorghum < sorghum < ordinary corn < wheat < rice < sago < arrowroot < potato < high-amylose corn. At 50 °C, salivary  $\alpha$ -amylase hydrolyses granular corn starch at one-eighth of the rate at which it hydrolyses the dissolved form [3]. However, there have been no published studies *in vitro* of enzymic hydrolysis of granular forms of amyloses with a low average degree of polymerization ( $\overline{dp} \approx 55$ –75) under conditions similar to those in the lumen of the human small intestine. Granular forms of this type are readily prepared in good yield from interaction of *Bacillus macerans* cyclodextrin

Key words:  $\alpha$ -cyclodextrin; cyclodextrin glucanotransferase; scanning electron microphotography; starch digestion; X-ray diffraction analysis.

Abbreviations used: CD,  $\alpha$ -cyclodextrin; CGTase, cyclodextrin glucanotransferase; dp, degree of polymerization;  $\overline{dp}$ , average degree of polymerization; SLM, scanning electron microphotograph.

glucanotransferase (CGTase) with  $\alpha$ -cyclodextrin (CD) over a wide range of temperatures [4]. Because of the possible usefulness of such amyloses as components of low digestibility in processed foods, a study *in vitro* was initiated in this laboratory to determine the susceptibilities of both cooked and uncooked forms to attack by  $\alpha$ -amylase. Conditions of pH, temperature and enzyme concentration were chosen to resemble those conditions in the human small intestine.  $\alpha$ -Amylase has optimal activity in near-neutral media (pH 6.9–7.0), similar to the environment in the proximal bowel [5]. Each enzyme molecule requires at least one calcium ion for enzymic activity and to prevent its destruction in the human gut by proteolytic enzymes [6]. Chloride ion is also essential for the action of  $\alpha$ -amylases [7], the optimal concentration of  $\text{Cl}^-$  being  $\approx 0.01 \text{ M}$  [3]. When human pancreatic  $\alpha$ -amylase is not readily available for use in studies of starch hydrolysis, commercially accessible human salivary  $\alpha$ -amylase can be used as an adequate substitute. Various investigators have observed a close similarity between properties of the two enzymes [8,9]. Lee has reported that there is no significant difference in the rate of hydrolysis by salivary, pancreatic and mammary  $\alpha$ -amylases [10]. Preduodenal hydrolysis by salivary  $\alpha$ -amylase is believed to play only a minor role in the overall digestion of starch in adults. Contribution of salivary  $\alpha$ -amylase to enzyme activity in the intestinal lumen is small, partly because of its partial inactivation by gastric acid prior to movement of the enzyme into the duodenum. Nevertheless, in normal adults, as much as 11% of the total  $\alpha$ -amylase output in postprandial jejunal fluid is salivary [11].

## Experimental

### Materials

$\alpha$ -Amylase (EC 3.2.1.1) from human saliva (chromatographically purified; 2500 units/mg of solid, as determined by the Bernfeld method [12]) was obtained from Sigma (St. Louis, MO, U.S.A.). According to the Bernfeld method for determining activity, 1 activity unit of this enzyme liberates the equivalent of 1.0 mg of maltose ( $2.9 \times 10^{-3} \text{ mmol}$ ) from soluble starch in 3 min at pH 6.9 and 20 °C. However, at the higher temperature of 37 °C the effective activity is 1.3 times greater than that at 20 °C. In this laboratory it was determined that 1 activity unit as determined at 20 °C and pH 6.9 will liberate  $3.8 \times 10^{-3} \text{ mmol}$  of reducing saccharides [malto-oligosaccharides of degree of polymerization (dp) 2–7 and small amounts of glucose] in 3 min at pH 7.2 and 37 °C. CGTase (EC 2.4.1.19) from *B. macerans* was obtained as an aqueous solution (600 units/ml, pH 7, according to the method of Tilden and Hudson [13]) from Amano International Enzyme Co. (Troy, VA, U.S.A.). CD (8.8%  $\text{H}_2\text{O}$ ) was

Table 1 High-amylose starches of low molecular mass formed by the conversion of CD by CGTase

Data for all conversions other than that at 2 °C were taken from [4]. Amylose analysis was by starch iodine analysis [14]. High amylose starch prepared at 2 °C was formed by reaction of CD (22.4 g, anhydrous weight) with CGTase (300 units) for 46 days in aqueous solution (215 ml) at pH 7. Streptomycin sulphate (7 mg) was added to prevent microbial growth. A similar yield was obtained in only 3 days when a much larger amount of CGTase (4800 units) was employed. Ycd (wt%) refers to the percentage yield calculated on the basis of the anhydrous weight of the starting material.

Reaction temperature (°C)	Ycd (wt%)	Moisture content (%)	Amylose analysis	
			dp	Amylose (%)
2	54	11.3	61	104
25	53	13.1	63	99
40	65	14.3	65	96
50	68	13.4	69	98
60	78	13.5	69	98
70	72	9.4	71	94

from Anspec/Ohio (Columbus, OH, U.S.A.). Corn amylopectin (11.5%  $\text{H}_2\text{O}$ ) and soluble potato starch were from Sigma; waxy-maize corn starch (10.7%  $\text{H}_2\text{O}$ ) and ordinary corn starch (10.1%  $\text{H}_2\text{O}$ ) were from Cerestar U.S.A. (Hammond, IN, U.S.A.); potato starch (Avebe; 12.0%  $\text{H}_2\text{O}$ ), and Hylon VII (64% amylose; 11.1%  $\text{H}_2\text{O}$ ), a high-amylose corn starch, were from National Starch and Chemical Corp., Bridgewater, NJ, U.S.A. Corn amylose ( $\approx 100\%$ ; 6.4%  $\text{H}_2\text{O}$ ) was prepared at this Center (the National Center for Agricultural Utilization Research, Peoria, IL, U.S.A.) from corn starch by complexation with butan-1-ol. Low-dp amyloses were prepared by the conversion of CD with CGTase at temperatures ranging from 2 to 70 °C according to methods described earlier [4]. Typical yields and compositions of these amyloses are presented in Table 1. All starches were stored in a chamber maintained at constant relative humidity (31%). Saccharides, used as standards in HPLC analyses, were from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Streptomycin sulphate was from Sigma. For buffering purposes, a stock 0.8 M solution of Tris/HCl, pH 7.2, was prepared. Stock solutions of  $\alpha$ -amylase (2000 units/ml of 1 mM  $\text{CaCl}_2$  solution) were stored in a freezer. Water was distilled and deionized.

### Analytical methods

Low-molecular-mass saccharides (dp 1–7) were determined by HPLC on a DuPont Zorbax NH<sub>2</sub> column (4.6 mm  $\times$  250 mm) at 40 °C with acetonitrile/water (13:7, v/v) at 1.0 ml/min and with refractometric detection. The reference standard contained 0.8 mg of each anhydrous carbohydrate/ml of aqueous solution. Calculated yields of saccharides formed by interaction of  $\alpha$ -amylase with starch

were based upon the initial amount (in mmol) of anhydro-glucose residues in the substrate.

Low-dp starches were evaluated for both amylose content and dp by spectrophotometric measurement of their amylose-iodine complexes, using the analytical method of Knutson [14] and utilizing the relationships between dp, iodine-binding capacity and wavelength of maximum absorption ( $\lambda_{\text{max}}$ ) reported by Banks et al. [15]. Amylose contents of natural starches were obtained by similar means. Moisture contents of substrates were determined by heating weighed samples under vacuum at 115 °C for 2 h.

#### Procedure for enzymic hydrolysis

Into screw-capped culture tubes were placed weighed amounts of starch hydrate and water. A measured volume of stock Tris/HCl buffer (13  $\mu\text{l}/\text{ml}$  of reaction mixture) was added to each to impart a Tris concentration of 0.01 M and to ensure that a pH of 6.8–7.0 was maintained throughout enzymic hydrolysis. Cooking, when desired for certain studies, was accomplished at this stage by first immersing the capped tubes in a bath of boiling water (100 °C) for 30 min and then cooling the tubes rapidly to room temperature. After measured volumes of stock  $\alpha$ -amylase were added, the tubes were placed in a 37 °C shaker bath for desired periods of time. Reactions were stopped by immersing the tubes in a dry ice/acetone mixture. Reaction mixtures were kept frozen (at or below –30 °C) until time for analysis. No inactivation of  $\alpha$ -amylase was necessary. Prior to analysis, each frozen mixture was thawed rapidly and centrifuged briefly at 980 g. An aliquot of the supernatant was diluted to an appropriate volume (usually 10 ml/ml of supernatant), and filtered by syringe through a Millipore HV 0.45- $\mu\text{m}$  filter. A sample of filtered solution was immediately injected into an HPLC equipped for saccharide analysis. The time lapse between centrifugation and HPLC injection was approximately 5 min.

#### Determination of $\alpha$ -amylase activity at 37 °C

Into a capped culture tube was placed 10.0 ml of a 0.08% (w/v) solution of soluble potato starch containing  $\text{CaCl}_2$  (1 mM), Tris/HCl (5 mM), and sufficient NaOH to give a pH of 7.2. After the tube was placed in a 37 °C water bath, a measured amount of  $\alpha$ -amylase (0.4 unit of activity based upon a Bernfeld assay at 20 °C) was introduced. At hourly intervals a 1-ml aliquot was removed, cooled quickly to room temperature, filtered by syringe through a 0.45- $\mu\text{m}$  filter, and subjected immediately to HPLC analysis for saccharides produced during enzymic hydrolysis. The time lapse between removal of sample from the bath and its injection into an HPLC column was 4–6 min. Saccharides of dp > 7 were not observed. Graphs of time versus mmol of total saccharides (dp 1–7) and time versus mmol of total

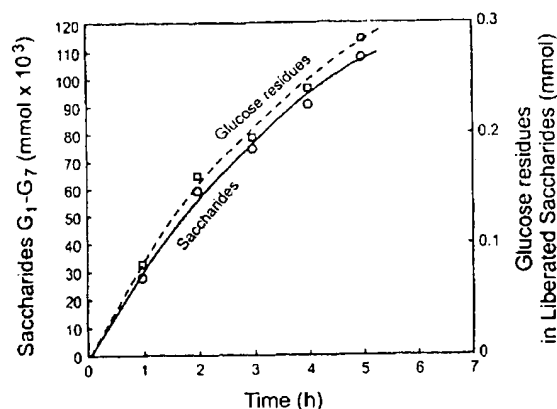


Figure 1 Hydrolysis of soluble potato starch by human salivary  $\alpha$ -amylase at 37 °C and pH 7.2

Initial starch solution (1.0 ml) contained 80 mg of soluble starch (anhydrous basis;  $\approx 0.493$  mmol of glucose residues) and was in 0.1 M  $\text{CaCl}_2$  and 5 mM Tris/HCl buffer. Human salivary  $\alpha$ -amylase was added to provide an activity of 0.04 unit/ml (based upon a Bernfeld assay at 20 °C [12]). Liberated saccharides (G1–G7) were determined by HPLC.

glucose residues in the combined saccharides (Figure 1) permitted a determination of activity not only in terms of total reducing groups produced during the first 3 min of reaction, but also in terms of total glucose residues produced. Liberated glucans appeared to be primarily, if not entirely, straight-chain malto-oligosaccharides, as indicated by their chromatographic retention times. The study revealed that  $\alpha$ -amylase activity (in terms of mmol of reducing groups produced) increased by a factor of 1.28 when the temperature was raised from 20 to 37 °C. Thus, at 37 °C and pH 7, a starch solution (10 ml) containing 0.4 unit of  $\alpha$ -amylase (based upon a Bernfeld assay at 20 °C) yielded 0.0015 mmol of reducing groups in 3 min. Had the reaction been conducted at 20 °C, only 0.00118 mmol of reducing groups would have been produced. At 37 °C during the first 3 min of reaction, 0.00415 mmol of glucose residues was liberated in the form of saccharides of dp 1–7.

## Results and discussion

Studies *in vitro* of starch hydrolysis, designed to provide information about starch digestion in humans, necessitated the use of conditions of temperature, pH, reaction time and  $\alpha$ -amylase activity associated with digestion in the intestinal lumen. An assumption was made that the average time for passage of food through the small intestine is 8 h. By means of calculations based upon published data, an  $\alpha$ -amylase activity of 13 units/ml of reaction mixture was chosen to represent a typical postprandial activity for intestinal contents. In pancreatic secretion,  $\alpha$ -amylase activity at 37 °C has been reported to be about 240 units/ml [9]; and the rate of

Table 2 Reaction of salivary  $\alpha$ -amylase at 37 °C and pH 7.0 with uncooked starches

Conditions:  $\alpha$ -amylase, 1.3 units/reaction; volume of mixture, 1 ml; starch, 40 mg (anhydrous weight; equivalent to 0.2467 mmol of glucose residues); 1 M/HCl buffer, 0.01 M;  $\text{CaCl}_2$ , 1 mM. Abbreviations: G<sub>0</sub>, D-glucose; G<sub>2</sub>, G<sub>3</sub>, straight-chain malto-oligosaccharides of dp 2–4; G<sub>5</sub>, G<sub>7</sub>, formed only in reactions where the substrate contained an appreciable amount of amylopectin, were probably branched glucans of dp 5, 6 and 7, respectively. Their HPLC elution times were significantly greater than the corresponding elution times of maltopentaose, maltohexaose and maltoheptaose. All starches but corn amylose were granular.

Reaction no./starch	Time (h)	Saccharide yields (nmol of G <sub>i</sub> residues × 10 <sup>3</sup> )								Degradation of starch to	
		G <sub>0</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>	Combined	G <sub>0</sub>	G <sub>7</sub> (%)
Natural starches											
1. Corn, ordinary (≈ 25% amylose)	2	4.9	35.4	2.3	0.5	0.6	1.5	2.2	66.4	26.9	
2. Corn, ordinary (≈ 25% amylose)	8	1.5	74.2	28.5	0	8	3.8	3.4	123.2	49.9	
3. Corn, ordinary (≈ 25% amylose)	20	16.7	114.8	23.4	0	1.7	3.7	3.3	163.6	66.3	
4. Waxy maize (99–100% amylopectin)	8	10.3	88.0	42.6	0	7	8.4	3.5	154.5	62.6	
5. Hylon VI (hybrid corn; 64% amylose)	8	3.4	19.0	3.0	0	0	0	0	25.4	10.3	
6. Amylose, 100% (non-granular; from corn)	8	6.7	62.6	17.7	0	0	0	0	87.0	35.3	
7. Potato	8	7.7	15.2	2.0	0	0	2.0	0	26.9	10.9	
8. Potato	20	8.9	24	0.9	Trace	0.5	1.0	0.5	35.9	4.6	
Amyloses, CD-derived, low dp											
9. 2 °C product	8	2.8	13.1	1.5	Trace?	0	0	0	17.4	7.1	
0. 25 °C product	8	4.5	4.4	2.3	0	0	0	0	21.2	8.6	
1. 50 °C product	8	3.9	11.6	2.1	0	0	0	0	17.6	7.1	
2. 50 °C product	26	6.8	13.8	0	0	0	0	0	20.6	8.4	
3. 70 °C product	8	6.4	23.2	5.4	0	0	0	0	35.0	13.5	

flow of this secretion into the duodenum is as high as 1200 ml/day, or 50 ml/h [7]. In the present study, an assumption was made that an average meal is consumed in 1 h and that the ultimate volume of ingested food and drink (including 50 ml of pancreatic juice) is 900 ml. Thus, an upper level of  $\alpha$ -amylase activity in the luminal contents would be (240 units/ml  $\times$  50 ml of secretion)/900 ml of luminal contents, or 13 units/ml. In several experiments, time and enzyme concentration were varied to provide information about the influence of such variations on the extent of hydrolysis. Salivary  $\alpha$ -amylase was employed as a substitute for pancreatic  $\alpha$ -amylase in all of the studies.

#### Hydrolysis of uncooked starches

All of the starches used in these studies were granular, with the exception of 100% corn amylose which, because of the method employed in its isolation from ordinary corn starch, was amorphous in appearance under a scanning electron microscope. Table 2 presents data on uncooked natural (native) starches (reactions 1–8) as well as on uncooked low-dp amyloses (reactions 9–13) prepared synthetically in this laboratory and described in Table 1. Among the granular natural starches, only Hylon VII and potato starch possessed low susceptibility towards attack by  $\alpha$ -amylase. The moderately high susceptibility of 100% corn amylose was probably related, at least in part, to its non-granular structure. X-ray diffraction analysis of this amylose revealed a V pattern, a type normally associated with amylose complexes in which guest molecules are bound within the tunnel-like cavities of starch-chain helices. The complexant butan-1-ol, which had been used in the isolation of amylose

Figure 2 SEM of ordinary corn-starch granules ( $\times 470$ )

from corn starch, was not present in the amylose, since it had been removed by azeotropic distillation at the conclusion of the isolation process. Examination of the amylose by solid NMR did not reveal the presence of any complexant. Yet, the V pattern suggested strongly that although the conditions of azeotropic distillation were sufficient for removal of complexant, they were not sufficient to allow extensive formation of 'crystalline' amylose, a spherulitic form of starch known to possess a B-type structure and to be highly resistant towards  $\alpha$ -amylase [16]. An X-ray diffraction pattern of type B was obtained for Hylon VII, whose granules have smooth surfaces that offer considerable resistance to enzymic attack (Table 2; reaction 5). Low-dp amylose granules derived from CD at 2–60 °C also have

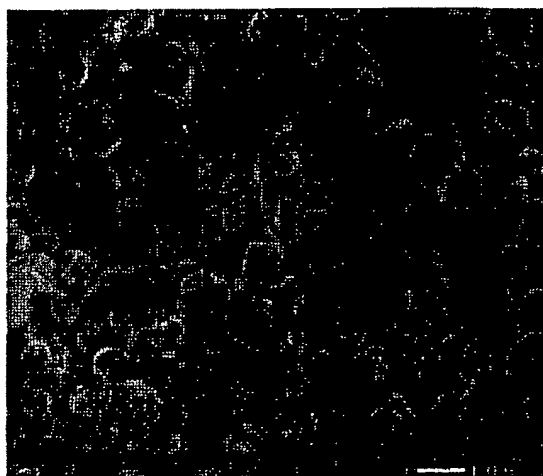


Figure 3 SEM of ordinary corn-starch granules after treatment in aqueous solution with salivary  $\alpha$ -amylase at 37 °C ( $\times 500$ )

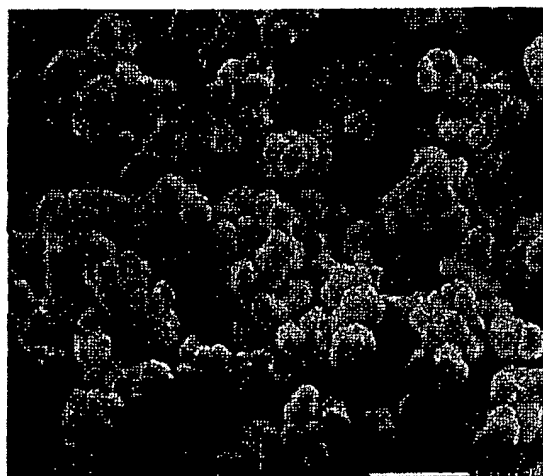


Figure 5 SEM of CD-derived amylose granules prepared at 25 °C ( $\times 1000$ )

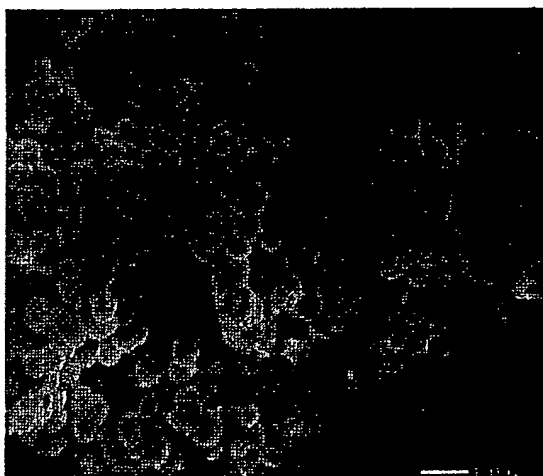


Figure 4 SEM of CD-derived amylose granules prepared at 2 °C ( $\times 500$ )

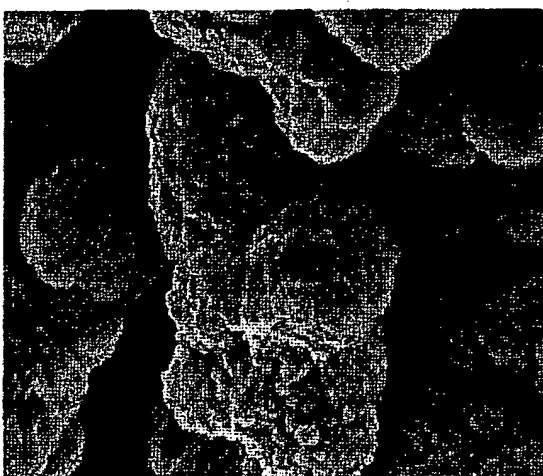


Figure 6 SEM of CD-derived amylose granules prepared at 25 °C ( $\times 5000$ )

type-B diffraction patterns. Their solubility in water is low and their resistance to  $\alpha$ -amylase is similar to that of Hylon VII granules and potato-starch granules.

For the natural starches listed in Table 2, the order of increasing resistance to  $\alpha$ -amylase is the same as that found by Leach and Schoch [2]. The positions of low-dp amyloses in this series are generally slightly higher than that of potato starch: waxy maize < ordinary corn < corn amylose (isolated from corn starch) < potato ~ Hylon VII < CD-derived amyloses. The relatively high reactivity of waxy maize and ordinary corn starch was expected because of the results of earlier studies by Fannon and co-workers [17,18], who investigated surface pores that occur on granules of corn, sorghum and millet starches, but not on granules

of potato, rice, wheat, oat, tapioca, arrowroot and canna. Those investigators proposed that the pores, which appear to be openings that lead to serpentine channels that penetrate into the granule interior, allow access of hydrolysing enzymes and thereby affect the pattern of enzymic attack. Figure 2 is a scanning electron microphotograph (SEM) of corn-starch granules, several of which have surface pores. Figure 3 is an SEM illustrating the extreme pitting that results from  $\alpha$ -amylase attack on corn-starch granules. Such pitting has not been observed with granules of CD-derived amyloses. Figures 4, 5 and 6 are SEMs of typical uncooked CD-derived granules. Their shapes are globular and their surfaces are rough, apparently the result of a binding



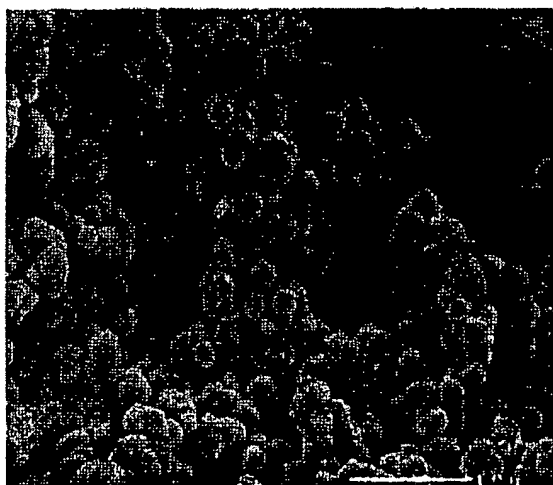


Figure 7 SEM of uncooked CD-derived amylose granules (prepared at 50 °C) that have been treated in aqueous solution with salivary  $\alpha$ -amylase at 37 °C ( $\times 1250$ )

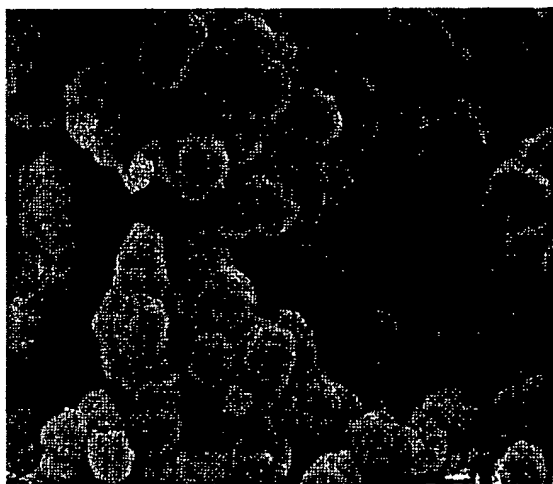


Figure 8 SEM of uncooked CD-derived amylose granules (prepared at 50 °C) that have been treated in aqueous solution with salivary  $\alpha$ -amylase at 37 °C ( $\times 2500$ )

together of numerous very small agglomerates to form much larger agglomerates, or granules. Figures 7 and 8 are SEMs of uncooked CD-derived amylose (prepared at 50 °C) that has been treated with  $\alpha$ -amylase. The enzyme caused little change in the spherical character of the granules.

Granules of CD-derived amyloses prepared at low temperature (2–25 °C) were, in general, totally birefringent under a polarizing microscope. However, birefringence occurred only sporadically among granules prepared at 50–70 °C, which would suggest that high preparative temperatures might be detrimental to orderly arrangements

of starch molecules in granular structures. It is not known at this time whether any relationship exists between granular birefringence and susceptibility of granules to enzymic hydrolysis.

#### Hydrolysis of cooked starches

Table 3 presents results of reactions of salivary  $\alpha$ -amylase at 37 °C with starches that have been cooked in aqueous media for 30 min at 100 °C. During cooking, granular starches from ordinary corn, waxy maize or potato become swollen and undergo extensive disruption with concomitant liberation of starch molecules. This process, commonly called gelatinization, greatly lowers the resistance of starch to enzymic attack. Even when cooking merely causes swelling, rather than disruption, of starch granules, as with hybrid corn starches of relatively high amylose content (e.g. Hylon VII and Amylomaize VII), there could be significant diffusion of starch molecules from the swollen granules into the surrounding medium. The magnitude of this diffusion might be large enough to have caused the moderately high levels of enzymic interaction reported in Table 3 (reactions 4, 5, 6 and 7), although not so high as the levels observed for cooked starches from ordinary corn, waxy maize and potato (Table 3; reactions 1, 2, 3, 10 and 11). Consideration should also be given to the possibility that cooking alters the structure of granules from Hylon VII and Amylomaize VII and thereby promotes their penetration by  $\alpha$ -amylase.

Cooked 100% corn amylose (Table 3; reaction 8) was hydrolysed by  $\alpha$ -amylase to approximately the same extent (34.7%) in 8 h as was the uncooked form (35.3%) reported in Table 2. Increasing both reaction time and enzyme concentration had almost no effect on extent of hydrolysis (Table 3; reaction 9). Cooking apparently had little effect, if any, on the structure of the starch.

Low-dp, CD-derived amyloses did not gelatinize when cooked in water at 100 °C. Dissolution in water at that temperature was only slight, being greatest with amylose prepared at 2 °C. Most of what dissolved at 100 °C precipitated in granular form very slowly over a period of hours when the mixtures were allowed to stand at room temperature. Reactions with  $\alpha$ -amylase at 37 °C were initiated immediately after cooking operations to minimize the lessening effect that precipitation of amylose could have on the extent of hydrolysis. All of the cooked CD-derived amyloses (Table 3, see reactions 12–17) were hydrolysed much less effectively than cooked granules of natural starches (Table 3, see reactions 1–7, 10 and 11). Those amyloses prepared at 50 and 70 °C were the most resistant, averaging 22.6% degradation after an 8-h reaction in which the  $\alpha$ -amylase activity was 13 units/ml of reaction mixture. In comparison, cooked starches of ordinary corn, waxy corn, Hylon VII, Amylomaize VII and potato underwent 81.8,

Table 3 Reaction of salivary  $\alpha$ -amylase at 37 °C and pH 7.0 with cooked starches

Cooking was conducted at 100 °C for 30 min. Conditions: volume of mixture, 1 ml; starch, 40 mg (anhydrous weight; equivalent to 0.2467 mmol of glucose residues); Tris/HCl buffer, 0.01 M;  $\text{CaCl}_2$ , 1 mM. Abbreviations: G, D-glucose;  $G_2$ ,  $G_n$ , straight chain malto-oligosaccharides of dp 2, 4;  $G_3$ ,  $G_n$ , formed only in reactions where the substrate contained an appreciable amount of amylopectin, were probably branched glucans of dp 5, 6 and 7, respectively. Their HPLC elution times were significantly greater than the corresponding elution times of maltopentaose, maltohexaose and maltoheptaose. In reaction 11 a component of molecular mass greater than that of  $G_4$ , probably  $G_5$  (dp 8), was indicated by HPLC analysis. Its contribution to overall yield of saccharides was estimated to be 2.8 mmol of glucose residues, which would raise the level of starch degradation to 77.8%.

Reaction no./starch	$\alpha$ -Amylase (units)	Time (h)	Saccharide yields (mmol of G <sub>n</sub> residues $\times 10^3$ )								Degradation of starch to	
			G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>	Combined	G <sub>1</sub>	G <sub>2</sub> (%)
Natural starches												
1. Corn, ordinary ( $\approx$ 25% amylose)	13	2	9.7	108.3	62.4	0	0.8	3.1	5.0	89.3		76.7
2. Corn, ordinary ( $\approx$ 25% amylose)	13	8	14.2	124.8	51.0	0	1.5	5.4	4.9	201.8		81.8
3. Waxy maize (99 : 100% amylopectin)	13	8	8.6	19.4	48.9	0	4.5	13.8	0	195.2		79.1
4. Hylon VII (hybrid corn: 64% amylose)	13	8	6.3	95.8	36.6	0	0	0	0	138.7		56.2
5. Hylon VII (hybrid corn: 64% amylose)	13	20	17.7	122.3	14.7	0	0.8	1.6	0	157.1		63.7
6. Hylon VI (hybrid corn: 64% amylose)	51	26	21.6	134.3	1.0	0	0	0	0	156.9		63.6
7. Amylomaize VII (hybrid corn: 66% amylose)	13	8	16.3	102.4	30.3	Trace	0.9	1.5	0	151.4		61.4
8. Amylose, 100% (non-granular: from corn)	13	8	6.1	60.6	18.9	0	0	0	0	85.6		34.7
9. Amylose, 100% (non-granular: from corn)	51	26	13.8	78.0	0.9	0	0	0	0	92.7		37.6
10. Potato	13	8	10.8	141.6	50.7	0	2.2	7.8	5.5	218.6		88.6
11. Potato	13	20	17.9	157.2	25.7	0	2.0	7.0	4.7	214.3		86.9
Amyloses, CD derived, low dp												
12. 2 °C product	13	8	4.8	49.0	3.8	0.6	0	0	0	68.2		27.6
13. 75 °C product	13	8	5.1	64.2	2.0	0	0	0	0	90.3		36.6
14. 40 °C product	13	8	7.2	57.8	14.4	0	0	0	0	79.4		32.2
15. 50 °C product	13	8	4.7	39.6	8.1	0.7	0	0	0	53.1		21.5
16. 50 °C product	13	20	7.3	36.2	1.8	Trace?	0	0	0	46.1		18.7
17. 70 °C product	13	8	4.5	44.4	9.6	0	0	0	0	58.5		23.7

79.1, 56.2, 61.4 and 88.6% degradation, respectively, under the same conditions. Among the CD-derived amyloses, observed variations in resistance to  $\alpha$ -amylase were possibly related, at least partly, to differences in granule solubility. Those amyloses with the higher solubilities would be expected to exhibit the greater susceptibilities to hydrolysis. Studies now in progress indicate an inverse relationship between average chain length (dp) and solubility at 100 °C. This relationship might explain the higher resistance observed for amyloses prepared at 50–70 °C. Figure 9 is an SEM of a CD-derived amylose (prepared at 70 °C) that was cooked and subsequently treated with salivary  $\alpha$ -amylase. The treatment had no observable effect on granular shape and very little effect on granular size.

The levels of hydrolysis reported in Table 3 for cooked CD-derived amyloses represent maximum levels. Treatment of the cooked amyloses with  $\alpha$ -amylase at 37 °C was made before any significant retrogradation of dissolved amylose could occur subsequent to the temperature reduction from 100 to 37 °C. Any retrograded amylose would probably exhibit an amylolytic susceptibility as low as that of uncooked amylose. Granules of CD-derived amylose are possibly very similar in composition and physicochemical properties to so-called 'resistant starch', produced when ordinary starch gels or amylose gels are given prolonged treatment with  $\alpha$ -amylase or mixtures of  $\alpha$ -amylase and pullulanase. 'Resistant

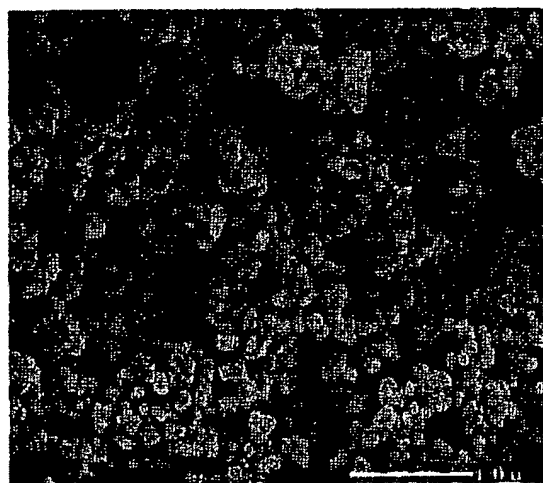


Figure 9 SEM of cooked CD-derived amylose granules (prepared at 70 °C) that have been treated in aqueous solution with salivary  $\alpha$ -amylase at 37 °C ( $\times 1250$ )

starch', prepared in this manner, is in the form of spherulites composed of highly organized linear glucans that possess a B-type X-ray diffraction pattern, a low solubility in water, and, frequently, a low dp ( $\approx$  20–60) [16,19].

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(54) **Biodegradable foamed plastic materials**

(57) Biodegradable foamed plastic material having a bulk density comprised within the range of from 5 to 13 kg/m<sup>3</sup>, comprising:

-- 30-99.5 by weight of polisaccharide containing more than 50% of thermoplastic starch or starch complexed by polymeric structures, having an intrinsic viscosity (in DMSO) lower than 1.3 dl/g and less than 20% by weight of soluble fraction in ethanol;

-- 0.5-70% by weight of a thermoplastic polymer which is soluble in starch, or is capable of complexing it;

-- 0-20% by weight of water.

The material can be used as a loose filler or for other foamed articles.

**EP 0 696 611 A2**

## Description

The present invention relates to biodegradable, foamed plastic materials and to the process for preparing them.

In the sector of foamed materials, used in particular, for protective packages, the need is more and more felt for replacing foamed polystyrene with materials meeting the biodegradability requirement.

Thanks to their biodegradability and antistatic properties, starch-based materials can represent a valuable alternative.

Unfortunately, the use properties of these products are not yet comparable to those of polystyrene in terms of costs, brittleness, trend to release dust, too high density values.

European patent application EP-A-087 847 discloses a process for preparing foamed, starch-based articles by heating starch or starch containing materials in an extruder in the presence of 10-30% by weight of water and a foaming agent, and subsequent extrusion.

European patent application EP-A-375 831 discloses foamed articles formed of high-amylose starch, displaying good mechanical characteristics and a closed-cell structure.

These foamed articles are prepared by extrusion in the presence of water at temperatures comprised within the range of from 150 to 250°C, optionally followed by a thermoforming treatment. High costs and brittleness under low humidity conditions are the drawbacks of this product.

Published international application WO 91/02023 discloses foamed articles of biodegradable plastic material prepared by extruding a composition which contains starch and a synthetic polymer selected from ethylene/vinyl alcohol and ethylene/acrylic acid, copolymers also in the presence of a polymeric acid and sodium bicarbonate as foaming agent. In this case, the products display an extremely high resilience and low density values, however their high cost renders them not competitive as loose-fillers.

International patent application WO92/18325 claims water dispersible foamed materials manufactured by starting from cereal and legume meals with low contents of amylose, submitted to partial dextrinization by intense mechanical working and/or presence of acids. Also the addition of polymeric agents, such as polyvinyl alcohol, is provided in a second step of the production process, in order to prevent the materials from undergoing degradation.

Although it is cheap, this product results to be brittle immediately after extrusion and must be wetted in order to increase its toughness. The bulk density reached in the case of "C"-shaped bodies is comprised within the range of from 8 to 15 Kg/m<sup>3</sup>.

International patent application 92/08759 relates to starch also containing up to 50% of cellulosic residues and small levels of natural rubber. The products according to that invention have bulk density values comprised within the range of from 11 to 36 Kg/m<sup>3</sup>, and are obtained by direct extrusion on an extruder with an L:D ratio of approximately 2.5; heat is supplied by friction.

US patent 5,185,382 relate to loose fillers which contain starch and polyethylene glycol obtained via direct extrusion, with bulk density values comprised within the range of from 10.8 to 36 Kg/m<sup>3</sup>.

It has now unexpectedly been found that a starch-based biodegradable foamed plastic material can be prepared which can be used as loose fillers and generally as a packaging material, in form of sheet or small-sized bondable particles or of injected foamed material, endowed with good toughness characteristics also under low moisture conditions, and with low values of bulk density. Thanks to its low bulk density values, comprised within the range of from about 13 to 5 Kg/m<sup>3</sup>, corresponding to a specific density lower than 32 kg/m<sup>3</sup>, the material results to be of low cost.

The foamed material according to the present invention comprises, as essential components:

-- from 30 to 99.5%, and preferably from 60 to 95% by weight of a polysaccharide containing an amount higher than 50%, of a thermoplastic starch which is plasticized or complexed by natural or synthetic polymeric structures, wherein said starch has:

-- an intrinsic viscosity in (dimethylsulphoxide) DMSO lower than 1.3 dl/g;  
-- an ethanol soluble fraction at 25°C lower than 20% by weight, preferably lower than 10% and still more preferably lower than 5%;

-- from 0.5 to 70%, preferably from 5 to 40% by weight, of one or more thermoplastic polymers, wherein at least 10% of this fraction is constituted by a thermoplastic polymer (A) which is soluble in starch, or (B) is capable of complexing starch; and

-- from 0 to 20%, preferably from 5 to 17% by weight of water.

The relationship existing between bulk density (weight of a defined volume of loose fillers) and specific density (pycnometric density as measured by using small glass spheres) is shown in Figure 1. As already stated, the specific density is lower than 32 kg/m<sup>3</sup> and can reach as low values as 18.5 kg/m<sup>3</sup>, or even lower.

The foamed materials according to the present invention have closed-cell structure.

The presence of one or more thermoplastic polymers which are soluble in starch, or are capable of complexing the starch in the composition of the foamed materials, allows to obtain a homogeneous, high melt strength molten mass and therefrom foamed articles which show good resilience and low moisture sensitivity.

The loose fillers useable in packaging industry can be given any shapes, including alphabetical characters, star shapes, cylindrical shapes and others.

The thermoplastic starch useable for preparing the foamed material according to the present invention can be a native starch, preferably maize, potato, tapioca, rice, wheat, pea starch, and also high-amylose starch, preferably containing more than 30% by weight of amylose, and waxy starches.

Furthermore, physically and chemically modified starch grades can be in part used, such as ethoxylated starches, oxypropylated starches, starch acetates, starch butyrate, starch propionates, with a substitution degree comprised within the range of from 0.1 to 2, cationic starches, oxidized starches, crosslinked starches, gelled starches, starches complexes by polymeric structures, characterized by a band at  $947\text{ cm}^{-1}$  when analyzed by second-derivative FTIR (Fourier Transform Infra Red analysis). The starchy material is converted into the thermoplastic state by treatment in heated extruders or any devices capable of securing temperature and shear conditions capable to cause the material to become thermoplastic, operating in the presence of water and/or a plasticizer at temperature from  $80^{\circ}\text{C}$  to  $210^{\circ}\text{C}$ .

The term "polysaccharide" as used herein, is meant to encompass not only refined starch, but also grains containing both starch and cellulosic products.

Independently on the starting starch used, the foamed material is characterized by a starch component having an intrinsic viscosity in DMSO lower than  $1.3\text{ dl/g}$  and preferably comprised within the range of from  $1.1$  to  $0.5\text{ dl/g}$ . Such a decrease in viscosity has however to take place without generating a high content of ethanol soluble sugars.

The absence of large amounts of low molecular weight fractions is responsible for the higher toughness of the foamed material already immediately after their extrusion.

The thermoplastic polymers useable as components of foamed particles are selected from :

i. polymers of natural origin, which may be either modified or non-modified, in particular, cellulose derivatives, as cellulose acetate, cellulose propionate, cellulose butyrate and their copolymers, having a substitution degree comprised within the range of from 1 to 2.5, optionally plasticized with caprolactone, low molecular weight polycaprolactone, or mono-, di- and tri-acetine, phthalate, in particular dimethyl phthalate, propane diol, polymers such as alkyl-cellulose hydroxyalkylcellulose, carboxyalkylcellulose, in particular, carboxyl methyl cellulose, nitrocellulose and, in particular, carboxyl methyl cellulose, nitrocellulose, and, furthermore, chitosan, pullulan or casein and caseinate, gluten, zein, soy bean proteins, alginic acid and alginate, natural rubbers, polyaspartates.

ii. biodegradable polymers of synthetic origin or from fermentation, in particular polyesters, such as homopolymers or copolymers of aliphatic  $\text{C}_2\text{-C}_{24}$  hydroxy acids, or of their corresponding lactone or lactides, and furthermore polyesters derived from difunctional acids and aliphatic diols, e.g.:

-- poly (epsilon-caprolactone), its grafted or block copolymers, the reaction products of caprolactone oligomers or polymers with aromatic or aliphatic isocyanates, polyureas, copolymers with lactic acid, glycolic acid, with polyhydroxy butyrates and polyhydroxy butyrate-valerate;

-- polymers of lactic acid or of lactide, polymers of glycolic acid or of polyglycolide, copolymers of lactic acid or glycolic acid;

-- polyhydroxy butyrate or polyhydroxy butyrate-valerate and copolymers with other polyester;

-- polyalkylene succinates, and, in particular, polyethylene and/or polybutylene succinate, polyalkylene sebacate, polyalkylene azelate, polyethylene or polybutylene brassilate and their copolymers, optionally copolymerized with aliphatic or aromatic isocyanates, with their molecular weight which can be increased by means of chain extenders;

iii. polymers capable of interacting with starch forming complexes, i.e. polymers which contain hydrophilic group intercalated to hydrophobic sequences, identified as (B) polymers, as:

-- ethylene: vinyl alcohol copolymers containing up to 50% by weight, preferably 10-44% by weight, of ethylene units, oxidized ethylene/vinyl alcohol copolymers, or ethylene/vinyl alcohol copolymers terminated with fatty acids, or grafted with polycaprolactone, or modified with acrylic or methacrylic monomers and/or pyridinium;

-- ethylene: vinyl acetate copolymers, also partially hydrolysed;

-- ethylene: acrylic esters copolymers;

-- ethylene: acrylic esters: maleic anhydride or ethylene:vinyl acetate:glycidyl methacrylate terpolymers;

-- ethylene copolymers with unsaturated acids such as acrylic acid, methacrylic acid, crotonic acid, itaconic acid, maleic anhydride, in particular ethylene: acrylic acid copolymers containing 5-50% by mole, of units deriving from acrylic acid;

-- ethylene : vinyl acetate terpolymers totally or partially hydrolysed with acrylic acid or methacrylic acid or crotonic acid or itaconic acid;

-- aliphatic 6-6, 6-9 or 12 polyamides, aliphatic polyurethanes, random or block copolymers polyurethane/polyamide, polyurethane/polyether, polyurethane/polyester, polyamide/polyester, polyamide/polyether, polyester/polyether, polyurea/polyester, polyurea/polyether;

-- polycaprolactone-urethane in which the size of polycaprolactone block is comprised within the range of from 300 to 3000 as molecular weight, and in which used isocyanates are MDI (methylene-diphenyl-diisocyanate), toluene diisocyanate, hexamethylene diisocyanate, toluene diisocyanate, hexamethylene diisocyanate, isophorone diisocyanate;

-- epoxy resins such as polyalkylene oxide/ester polymers ended with glycidil groups.

iv. soluble polymers, and anyway capable of forming hydrogen bonds with starch, identified as (A) polymers, in particular polyvinyl alcohol with various hydrolysis degrees, possibly modified with acrylate or methacrylates, polyvinyl alcohol previously plasticized or modified in order to decrease the melting point thereof, possibly containing geleating agents such as boric acid, borates or phosphates, copolymers of vinyl acetate in various hydrolysis degrees with vinyl pyrrolidone or styrene, polyethyl oxazolines, polyvinyl pyridine. Preferred thermoplastic polymers are polyvinyl alcohol, copolymers of an olefinic monomer, preferably ethylene, with a monomer selected from vinyl alcohol, vinyl acetate, acrylic acid and methacrylic acid, aliphatic polyesters, such as polycaprolactone, polybutene succinate and their copolymers, aliphatic polyamides and polyester-urethanes.

The foamed materials according to the present invention preferably contain a nucleating agent.

The amount of such a nucleating agent in the foamed material is comprised within the range of from 0.005 to 5% by weight, preferably of from 0.05 to 3% by weight, still more preferably of from 0.2 to 2% by weight.

Useable nucleating agents are, e.g., inorganic compounds such as talc (magnesium silicate), calcium carbonate, possibly with their surface being treated with adhesion promoters such as silanes, and titanates. Furthermore, organic fillers can be used such as shells of yeasts coming from sugar beet processing, dried, ground and powdered sugar beet flesh, wood dust, cellulose powder and fibrils.

The nucleating agent can be added to the blend used to prepare the foamable pellets or, according to an alternative route, it can be added to the foamable pellets in master-batch form. In that case, said master-batch can contain amounts comprised within the range of from 10 to 50% by weight, of one or more fillers.

The foamable particles or the formulation to be foamed may furthermore contain one or more lubricant and/or dispersing agents having a hydrophilic/lipophilic balance index (HLB) comprised within the range of from 3 to 25, preferably of from 6 to 20. When they are used, these agents can be present in amounts comprised within the range of from 0.01 to 5% by weight, preferably of from 0.1 to 3% by weight.

The foamable pellets, or the formulation to be foamed, can also contain one or more plasticizer agents. When they are used, said plasticizer agents are present in amount comprised within the range of from 0.5 to 20% by weight, preferably of from 0.5 to 5.0% by weight.

Useable plasticizer agents are, for instance, those disclosed in published international patent application WO92/14782, the content of which is understood as being incorporated to the present disclosure by reference. Glycerol, sorbitol, mannitol, erythritol, low molecular weight polyvinyl alcohol, besides the oxy-ethylated or oxy-propylated derivatives of said compounds, and furthermore urea, are particularly suitable.

The compositions can also contain compounds of polyvalent elements belonging to the III and IV Groups of the Periodic Table, preferably belonging to the III Group, and preferably in the presence of oxygen.

Such compounds include: boric acid, boric anhydride, sodium metaborate, both anhydrous and hydrated sodium borate, alumina hydrated alumina, aluminium carbonate, aluminum acetate and still other carboxylates, aluminum borate, aluminum phosphate, sodium-aluminum phosphate, silic acid, alkali and alkaline-earth metal aluminates, as sodium silicate and magnesium silicate.

Furthermore, the foamable pellets or the formulation to be foamed may contain one or more flame retardants, which can be added to the blend used to prepare the foamed particles or, according to an alternative route, can be added to the foamed particles in a master-batch form, in particular in combination with the nucleating agent.

When they are used, these flame retardants are present in amounts comprised within the range of from 0.1 to 20% by weight, preferably of from 1 to 10% by weight, still more preferably of from 2 to 5% by weight.

Useable flame retardants can be selected from those derived from phosphorus, sulfur or halogen containing products, or combinations thereof. For exemplifying purposes, triphenyl phosphate, tributyl phosphate, tricresyl phosphate, tributoxyphenyl phosphate, melamine pyrophosphate, ammonium polyphosphate, ethylene diamine, ammonium polyphosphate, guanidium phosphate, tetrabromophthalic anhydride, halogenated paraffins, diphenyl oxide with different bromination degrees, ammonium sulfate, ammonium sulfamate, are suitable for the intended purpose. Particularly advantageous are ammonium sulfate, ammonium sulfamate, ammonium polyphosphate, guanidium phosphate and melamine pyrophosphate.

Still other flame retardants which can be used are aluminium hydroxide, antimony oxide, ammonium perborate, ammonium octamolybdate.

In the case of particular applications, the presence of substances displaying rodent repellent activity can be required in the foamable formulations. These substances can be added to the blend used to prepare the foamed materials, or they can be added to the foamed materials as microcapsules containing the active principle, or as a master-batch, in particular in combination with the nucleating agents and/or flame retardants.

For that purpose, such substances as N, N-diethyl-m-toluamide, diethyl phenyl acetamide, 2-decenal, ammonium chloride, potassium chlorate, terpenoids, cycloheximide, diguanidinoazaptadecane, can be used. Terpenoids and, in particular, menthol and limonene, are preferred.

When they are used, these rodent repellents are present in amounts comprised within the range of from 0.1 to 5% by weight, preferably comprised within the range of from 1 to 3% by weight.

The foamed material according to the present invention can be prepared by means of a two-step process, the first step of which consists of the direct extrusion of the starch-based composition, carried out by means of a single-screw or twin screw extruder, by operating at a temperature comprised within the range of from 150 to 200°C.

In this first step, the non-dried starch is extruded in the presence of an added water amount comprised within the range of from 5 to 20% by weight.

The extrusion causes starch to get thermo-plasticized and/or complexed owing to the joint effect of water, temperature, presence of specific thermoplastic polymers of (A) and/or (B) types. The total water content at the nozzle is comprised within the range of from 5 to 20% by weight, and the intrinsic viscosity of starch in DMSO is comprised within the range of from 1.5 to 8 dl/g in those cases in which starch is constituted by less than 50% of amylose. The total water content in the pellets is between 5 and 20% by weight.

The second step consists in foaming foamable pellets containing starch with an intrinsic viscosity in DMSO comprised within the range of from 2 to 8 dl/g, using a single screw extruder with a whatever ratio of screw length/screw diameter, equipped with a screw capable of yielding a specific energy higher than:

$$T-200$$

$$0.1 \text{ kWh/kg} - \text{-----} \times 0.01 \quad (1)$$

5

for residence times of the molten material longer than seconds where L/D is the length/diameter ratio of the screw and for temperatures (T) at the head of the extruder comprised within the range of from room temperature to 240°C.

If such conditions are verified, then a low-specific-density material, having a lower specific density than 32 kg/m<sup>3</sup> and a lower intrinsic viscosity than 1.3 dl/g is also obtained for residence times inside the extruder chamber comprised within the range of from 20 to 60 seconds.

A foaming equipment capable of developing the energy defined by equation (1) allows to obtain foamed materials having density lower than 32 kg/m<sup>3</sup> even felding granules prepared under pasting conditions i.e. under temperature conditions in the first step lower than 150°C and using grater amounts of water (from 12 to 30 w% of added water). Operating under the above pasting conditions the swelling of starch granules is obtained (gelling) without, however, distraing the starch structure. The water concentration is kept to the values optimal for the foaming with a final drying step.

The granules can also be prepared by aggregating starch and the other components just by effect of pressure, without using heat.

#### Method for determining solubility in alcohol

An amount of 1 gram of foamed product is dispersed in 30 ml of an aqueous solution containing 80% ethanol. After a 15 minutes stirring by means of a mechanical stirrer, the suspension is centrifuged at 180 revolutions per minute, for 10 minutes. The supernatant is decanted off, then is extracted once again. Sugars contained in supernatant are determined by means of anthrone method.

#### Method for determining intrinsic viscosity in DMSO

A volume of 10 ml of dimethyl sulfoxide (DMSO) is charged to the tank of a BISHOP viscometer, the so prepared instrument is then transferred to a thermostatic bath preset at 30°C, and approximately 30 minutes later, recording the pure solvent flow times is started. About 50 mg of sample is then weighed and is added to the cup of the viscometer;



10 ml of DMSO is added, the tank is closed with the suitable ground stopper and the whole preparation is kept stirred for 1 hour at 70-80°C. When dissolution is complete, the cup is applied to the viscometer, and the instrument is transferred to a thermostatic bath at 30°C. The flow times of the solution are recorded performing a plurality of readings and calculating the average flow time.

Through the purposely provided tube, the solution contained within the tank of the instrument is diluted with 10 ml of pure DMSO; the resulting diluted solution is allowed to reach temperature equilibrium and the flow times are recorded.

A further dilution is then carried out with a further volume of 10 ml of DMSO, and the whole process is repeated.

To compute the intrinsic viscosity, the concentration (c) values are reported on a chart versus the ratio of specific viscosity: concentration ( $\eta$  sp/c) and the resulting line is extrapolated to (C=0) to obtain the value of  $[\eta]$  (intrinsic viscosity).

$$\eta_{rel}$$

$$\eta_{sp}/c = \text{-----}$$

$$c$$

wherein

$$t_{solut.}$$

$$\eta_{rel} = \text{-----} - 1$$

$$t_{solv.}$$

c = concentration, expressed as polymer g per 100 ml of solution.

t solut. = average flow time of solution, as seconds.

t solv. = average flow time of pure solvent, as seconds.

#### Definitions

Specific density (kg/m<sup>3</sup>)      pycnometric density of the individual foamed bodies

Bulk density (kg/m<sup>3</sup>)      density value calculated from the weight of that amount of foamed elements which is necessary to fill a volume of 16 litres.

#### EXAMPLE 1

75 w.pts of potato starch, 9 w.pts of 87%-hydrolyzed polyvinyl alcohol having a molecular weight of 70000, 15 w pts of water and 0.1 w.pts of glycerol momooleate are fed to a twin-screw extruder of 60 mm of diameter, L/D=36.

The screw, which was constituted by an 8 diameter long zone of mixing elements, had a venting zone between diameters 29 and 32.

The head, which contained 24 bores of 3 mm of diameter, was thermostated at 120°C. The temperature profile used for the nine heating zones was the following: 90-110-175-175-175-175-130-120-120°C.

The revolution speed of the screw was of 120 rpm and the throughput was of the order of 40 kg/h.

A material resulted with intrinsic viscosity in DMSO of 3.1 dl/g and a water content of 15.7%.

#### Examples 2-5

The pellets obtained by operating according to as disclosed in Example 1 were fed to a single screw Ghioldi extruder of 40 mm of diameter with L:D=30, forced feed on a 4-zone thermostating (cooling by fans) equipped with a 20 mm long nozzle of 2 mm of diameter, equivalent to a ratio of L:D=10.

Two screw types were used for the several examples:

- compression ratio 1:3 with constant profile;
- compression ratio 1:3 with metering profile.

Before foaming, the foamable pellets were added with 0.5% of talc (average particle size = 1.3  $\mu\text{m}$ ).

The results are reported in Table 1.

From the obtained data, it clearly appears that in the case of the screw with constant compression ratio (examples 2C - 5C), capable of applying a specific energy not higher than 0.1 kWh/kg for a residence time of the molten material in the chamber of about 140 seconds, which does not comply with the conditions of formula (1), the density of foamed material for residence times inside the extruder chamber comprised within the range of from 40 to 66 seconds, increases with increasing screw revolution speed and is always higher than 32 kg/m<sup>3</sup>. Surprisingly, in the case of the metering screw (Examples 2-5), which already at a residence time inside the extruder chamber of 140 seconds applies a specific power higher than 0.1 kWh/kg, very low density values are obtained which tend to remain constant with varying throughput rates.

The products obtained with the metering screw are very resilient soon after foaming.

In Example 3, the soluble portion in an aqueous solution of ethanol at 80% is of 5%.

#### Examples 6-8

The used foaming conditions are similar to those as of Example 3C, with temperature being varied according to as reported in Table 2. One will observe that the increase in temperature causes a progressive decrease in bulk density, until lower values than 32 kg/m<sup>3</sup> are reached by starting from temperatures of the molten material of 215°C. The values of specific energy at residence times within the extruder chamber of 140 seconds by starting from 215°C result in fact to be higher than, or equal to, those as provided for by equation (1).

#### Example 9-11

The same conditions as of Example 5 were adopted, but with a different temperature profile, which was according to as reported in Table 2.

A progressive decrease in density can be clearly seen, which density remains always lower than 32 kg/m<sup>3</sup>. The specific energy at 140 seconds results to be always higher than the values given by formula (1).

#### Examples 12-13

The same conditions as of Example 3C-4C are repeated, however with the chamber volume being increased. The results are reported in Table 3.

In particular, a slight decrease in density can be observed, which is anyway insufficient to reach lower density values than 32 kg/m<sup>3</sup> within the examined residence time range.

#### Examples 14-18

The conditions for these Examples are reported in Table 3, and concern the change in extruder chamber volume, with all other conditions, relatively to Examples 3-5, being the same. One will observe that in this case, the increase in the residence times has a very meaningful effect on the decrease in density which, in some cases, approximates 21 kg/m<sup>3</sup>.

The corresponding bulk density reaches values of 6.5 kg/m<sup>3</sup>. All density data from the above examples correlate, in this system, with the intrinsic viscosity values displayed by the same materials, as measured in DMSO.

#### Example 19

The same composition as in Example 1, in which potato starch is replaced by a high-amylose starch, Eurylon VII, was processed according to the same conditions as in Example 1, and was then foamed according to the conditions as in Example 5, apart from the shape of the nozzle which, rather being circular, was "S"-shaped, with a surface area of 6.2 mm<sup>2</sup>.

Starch intrinsic viscosity, foamed material specific density and bulk density were found to be of 0.67 dl/g, 18.8 kg/m<sup>3</sup>, and 5 kg/m<sup>3</sup>, respectively.

Example 20

A composition is taken into consideration which is only different from the composition of Example 1, for the replacement of polyvinyl alcohol with polycaprolactone-urethane Estane 54351, containing polycaprolactone units with a molecular weight of 530 and urethane units deriving from methylene diphenyl diisocyanate.

Under the same conditions as of Example 5, a foamed product was obtained which contains starch having an intrinsic viscosity in DMSO of 1 dl/g, and has a specific density of 25 kg/m<sup>3</sup> and a bulk density of 7 kg/m<sup>3</sup>.

Example 21

84.5 w.pts of wheat starch, 14 w.pts of polyvinylalcohol 0.4 w.pts of glycerol-monostereate, 1.6 w.pts of water 0.5 w.pts of talc were mixed in a slow mixer and fed to a pelletizer formed of grooved rotors pressing the mixture against a holed ring. The dimension of the pellets is regulated by a knife rotating around the ring.

The temperature increase during the extrusion was of 60°C-80°C due to friction.

The obtained pellets were foamed according to example 5.

The specific density was 25.9 kg/m<sup>3</sup> and the intrinsic viscosity 1.29 dl/g.

Table 1 : Examples 2-5

Example Nos.	Screw	Specific energy at a residence time in the extruder chamber of 140 seconds (kWh/kg)	Screw revolution speed, rpm	Feed flow rate (kg/h)	Melt (°C)	Residence time inside extruder chamber (seconds)	Characteristic of foamed material	
							Specific density (kg/m <sup>3</sup> )	Intrinsic Viscosity $\eta$ (in DMSO) (dl/g)
2 C+	C.T. CR 1:3	0.085	90	46.9	195	66	33.6	1.4
3 C+		0.085	110	57.5	195	53	37.3	
4 C+		0.085	130	69.7	199	44	38.4	
5 C+		0.085	150	79.4	200	39	40	1.56
2	METERING CR 1:3	0.12	90	34.1	200	77	25.6	1.1
3		0.12	110	41.1	197	64	25.7	1.28
4		0.12	130	48.9	197	53	25.1	
5		0.12	150	56.8	202	46	24.9	1.31

C' = Comparison Example

CR = Compression ratio

Table 2 : Examples 6-11

Example Nos.	Screw	Specific energy at a residence time in the extruder chamber of 140 seconds (kWh/kg)	Screw revolution speed, rpm	Feed flow rate (kg/h)	Melt (°C)	Residence time inside extruder chamber (seconds)	Characteristic of foamed material	
							Specific density (kg/m <sup>3</sup> )	Intrinsic Viscosity ( $\eta$ DMSO) (dl/g)
3 C+	C.T. CR 1:3	0.085	110	57.5	195	53	37.3	
6 C+		--	110	58.5	210	53	34	
6		0.068	110	58.7	215	52	32	
7		--	110	58.6	220	52	29.8	
8		0.057	110	58.6	225	52	26.9	
5	METERING CR 1:3	0.12	150	56.8	198	46	24.9	1.31
9		0.1	150	58	210	45	25	
10		0.09	150	60	215	44	25	
11		--	150	60.5	225	43	24	

C\* = Comparison Example  
CR = Compression ratio

Table 3 : Examples 12-18

Example Nos.	Screw	Extruder volume (cm <sup>3</sup> )	Screw revolution speed, rpm	Feed flow rate (kg/h)	Melt (°C)	Residence time inside extruder chamber (seconds)	Characteristic of foamed material	
							Specific density (kg/m <sup>3</sup> )	Intrinsic Viscosity $\eta$ (in DMSO) (dl/g)
3 C+	C.T. CR 1:3	610.8	110	57.5	195	53	37.3	
4 C+		610.8	130	69.7	199	44	38.4	
12	C.T. CR 1:3	802.5	110	57.1	208	71	35.4	
13		802.5	130	67.9	209	59	34.3	1.45
3	METERING CR 1:3	518.6	110	41.1	197	64	25.7	1.28
4		518.6	130	48.9	197	53	25.1	
5		518.6	150	56.8	202	46	24.9	1.31
14	METERING CR 1:3	722.5	110	42	199	87	21.6	0.91
15		722.5	130	50	196	73	21.5	
16		722.5	150	57.5	198	63	22.3	0.94
17	METERING CR 1:3	790.5	110	43.5	210	91	21.6	
18		790.5	150	59.5	211	67	21.2	0.85

C' = Comparison Example

CR = Compression ratio

## Claims

1. Biodegradable foamed plastic material having a bulk density value comprised within the range of from 5 to 13 kg/m<sup>3</sup>, and a corresponding specific density equal or lower than 32 kg/m<sup>3</sup>, comprising:
  - from 30 to 99.5% by weight of a polysaccharide containing a level higher than 50%, of thermoplastic starch or starch complexed with natural or synthetic polymeric structures, wherein said starch has:
    - an intrinsic viscosity lower than 1.3 dl/g;
    - an ethanol soluble fraction at 25°C lower than 20% by weight;
  - from 0.5 to 70% by weight of one or more thermoplastic polymers, wherein at least 10% of this fraction is constituted by a thermoplastic polymer (A) which is soluble in starch, or (B) is capable of complexing starch; and
  - from 0 to 20% by weight of water.
2. Foamed material according to claim 1 in which starch has an intrinsic viscosity comprised within the range of from 1.1 to 0.5 dl/g and a content of ethanol soluble fraction lower than 20% by weight.
3. Foamed material according to claim 2, wherein the ethanol soluble fraction is lower than 10% by weight.
4. Foamed material according to the claims from 1 to 3, in the form of sheet or sinterable particles or injection moulded articles, or as foamed loose fillers useable for packaging.
5. Foamed material according to the preceding claims from 1 to 4, in which the thermoplastic polymer is selected from modified or unmodified natural polymers.
6. Foamed material according to claim 5 wherein the natural polymer is a cellulose derivative with a substitution degree comprised within the range of from 1 to 2.5.
7. Foamed material according to the preceding claims from 1 to 4, in which the thermoplastic polymer is selected from homopolymers and copolymers of aliphatic C<sub>2</sub>-C<sub>24</sub> hydroxy acids, their corresponding lactones and lactides and aliphatic polyesters deriving from bicarboxylic acids and aliphatic diols.
8. Foamed material according to the preceding claims from 1 to 4 and 7, in which the (A) thermoplastic polymer is selected from polyvinyl alcohol possibly modified with acrylates or methacrylates polyvinyl alcohol previously plasticized or modified in order to lower its melting point, copolymers of vinyl acetate with vinyl pyrrolidone or styrene, polyethyloxazolines or polyvinyl pyridine.
9. Foamed material according to the preceding claims from 1 to 4 and 7, 8, in which the (B) thermoplastic polymer is selected from copolymers of ethylene with vinyl alcohol or vinyl acetate or unsaturated acids, aliphatic 6-6, 6-9 or 12 polyamides, aliphatic polyurethanes, polyurethane-polyamide, polyurethane-polyether, polyurethane-polyester copolymers.
10. Foamed material according to the preceding claims from 5 to 9, in which the thermoplastic polymer is contained in an amount comprised within the range of from 2 to 35% by weight.
11. Foamed material according to the claims from 1 to 10 in which the complexed thermoplastic starch is obtained from native starch, comprising maize, potato, tapioca, rice, wheat or pea starch, and starch with an amylose content higher than 30% by weight.
12. Foamed material according to the claims from 1 to 4 and 7 to 10, in which the synthetic polymer is selected from polyvinyl alcohol, ethylene copolymers with a monomer selected from vinyl alcohol, vinyl acetate, acrylic acid and methacrylic acid, polycaprolactone, polybutylene succinate and copolymers thereof, aliphatic polyamides and polyester-urethanes.
13. Foamed material according to the claims from 1 to 12, containing an additive selected among the nucleating agents, the flame retardants and agents acting as a rodent repellent.
14. Process for preparing the foamed material according to the claims from 2 to 13, comprising the steps of

# CORN AND SORGHUM STARCHES: PRODUCTION

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## I. INTRODUCTION

The word "corn" is used in the United States as the common name for the cultivated member of the grass family (Gramineae) known to botanists as *Zea mays* L. More specifically, "corn" here means the seed produced by this plant. Outside of the United States, this crop is commonly known as maize. Corn is believed to be a product of domestication in Central Mexico beginning 5000–7000 years ago. The evidence is now quite strong that domestic corn was derived by human selection from mutants of the grass, teosinte (*Zea mays*, ssp. *Mexicana*) which grows wild in Mexican central highlands (1). Recent discovery of a distinct new species, *Zea diploperennis* Iltis (2), a wild perennial teosinte with the same number of chromosomes as corn has given new validity to the teosinte



origin. Corn has reached its present state of development through continual mutations, hybridizations, segregations, and selections by random, natural processes and conscious selection. By this process, a number of types have developed which differ primarily in structure of the seed. Examples are popcorn, sweet corn, dent corn, flint corn, and flour corn.

Corn has been the staple food for countless generations of Indians of North and South America. The diverse seed types were probably selected and cultivated by these primitive farmers in response to food preferences. "Mahys" specimens taken to Spain by Columbus on an early voyage, introduced corn to Europe. The first recorded planting of corn was near Seville in 1494 (3) from whence it subsequently spread to all of Europe, Asia Minor, and eventually to the Far East. It is interesting that a mutant of considerable industrial importance, waxy maize, was first discovered in China in 1909 (4). Flint types of corn gradually became an important crop across Southern Europe and Turkey. However, it was the development of American dent types and their eventual hybridization that propelled corn to the position of agricultural dominance as the most cost effective feed grain so important in development of today's highly specialized animal agriculture.

The ready availability of corn at relatively low and steady prices, its storability from season to season, and its high starch content led naturally to development of commercial processes for recovery of corn starch. From the early nineteenth century, when Yankee inventors first discovered that corn starch was fairly easily recovered by grinding the soaked grain, the process has gradually evolved into today's highly sophisticated automated process, which produces a multitude of useful food and industrial products. Mechanical innovations developed by trial and error during full-scale operations stimulated much of the early growth of the corn starch industry (5). Today, process and product improvements more commonly follow research and engineering studies and thorough pilot plant evaluations.

Process innovations have resulted from pressures to improve worker efficiency and work place environment, to reduce energy consumption, to reduce air and water pollution, to improve end-product quality, and to introduce new products. In the last 20 years, older facilities have been completely redesigned and expanded, and ten completely new wet milling facilities have been built in the United States since 1970. Such installations now require much less space than the units they replaced; working conditions and plant sanitation have vastly improved; continuous operation and product monitoring have improved product quality and uniformity.

Grain sorghum (*Sorghum bicolor* Moench) is a cereal grain also known in some localities as milo, milo maize, or kaffir corn. Sorghum culture probably began in Eastern Africa 5000 to 7000 years ago and spread to all of Africa, Europe, and Asia and eventually to the United States in the mid-nineteenth

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